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DRD2 and XKR4: A genetic approach to fescue toxicosis.

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I am submitting herewith a thesis written by Kristine Marie Ely entitled "DRD2 and XKR4: A genetic approach to fescue toxicosis.." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

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DRD2 and XKR4: A genetic approach to fescue toxicosis.

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Dedication

This thesis is dedicated to my dog Mickie. She may never understand what that means but no one else suffered through more endless nights analyzing statistics and writing, and I cannot be more thankful to have my faithful companion by my side through it all. I would also like to thank my family, specifically my grandparents, they are the reason I fell in love with Tennessee. My cousin Ashlee has always been my rock and provided many words of encouragement, for that I will be forever thankful.

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Abstract

Tall fescue (*Lolium arundinaceum Schreb*) is the most prevalent forage in the Mid-south United States due to its observed hearty nature and ability to withstand heavy grazing. This is due to a symbiotic relationship with an endophytic fungus (*Neotyphodium coenophialum*). This fungus produces ergot alkaloids, which can bind to many adrenergic, serotonergic, and dopaminergic receptors. One of the primary functions of dopamine is to inhibit prolactin response. Decreased growth, reproduction, lactation, and depressed serum prolactin concentrations are commonly observed in beef cattle grazing endophyte-infected tall fescue. These symptoms are collectively referred to as fescue toxicosis, and are estimated to cause over $600 million in losses to the beef industry annually. Polymorphisms in the Dopamine Receptor D2 (DRD2) and XK, Kell blood group complex subunit related family member 4 (XKR4) genes may modulate these responses. The objective of this study was to assess associations between each single nucleotide polymorphism (SNP) and serum prolactin concentration, hair coat score (HCS), body condition score (BCS), adjusted birth weight (ABW) and adjusted 205-day weight (A205) in fall calving beef cattle grazing endophyte infected tall fescue in Missouri. The results indicated that dam genotype for DRD2 and XKR4 combined were associated with serum prolactin concentrations ($p = 0.04$). Serum prolactin concentrations were greater in cows having 2 copies of the 'A' allele for DRD2 and at least 1 'A' allele for XKR4. Dam genotype for DRD2 was associated with HCS and BCS ($p = 0.0024$ and $p = 0.011$, respectively). The association between dam genotype

for DRD2 and hair coat score was similar to what has been seen previously. Dam genotype for DRD2 and XKR4 differed in ABW ($p < 0.0001$ and $p = 0.027$, respectively), such that dams with the 'AG' genotype for DRD2 and 'GG' genotype for XKR4 had increased ABW. Calf genotype for XKR4 was associated with A205 ($p = 0.011$); calves with the 'AA' genotype had increased A205 compared to calves with the 'AG' or 'GG' genotype. These results suggest that the use of these genetic markers could improve cow-calf production in beef cattle grazing endophyte-infected tall fescue.

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Chapter 1: Literature Review

Tall Fescue

Tall Fescue (*Lolium arundinaceum Schreb*) is a cool-season, perennial bunch grass that is the most prevalent forage in the Mid-south United States (Stuedemann J.A. 1988). Tall Fescue was introduced into the United States from Europe in the 1800s but its popularity did not develop until the 1940s when the variety Kentucky-31 was released (Hill *et al.* 1991). Kentucky-31 was cultivated due to its observed hearty nature and its ability to withstand heavy grazing, drought, pests, and disease (Hill *et al.* 1991). Tall fescue has both a fall and spring growing season; the spring growing season being from mid- March to late June and the fall growing season being from mid-September and ends early December (Ball *et al.* 1996). Due to these positive characteristics, tall fescue is heavily relied on as the primary forage option for cattle producers in the mid-south United States.

Tall fescue grows in what is commonly referred to as the fescue belt, which covers over 14 million hectares in the United States (Stuedemann J.A. 1988; Fribourg H.A. 1991a), primarily within the southern climate transition zone. This zone encompasses the southern portions of Illinois and Ohio, to the northern portions of Mississippi and Georgia. It spans westward to eastern Oklahoma, and eastward to the Piedmont of Virginia and the Carolinas (Fribourg H.A. 1991a). Tall fescue's resilience, which results from a symbiotic relationship with an endophytic fungus (*Neotyphodium coenophialum*), is the primary reason it is widely cultivated. This endophytic fungus produces ergot

alkaloids (Porter *et al.* 1979), which are beneficial to the plant but not advantageous to cattle upon ingestion.

Ergot Alkaloids

Symptoms associated with fescue toxicosis are attributed in part to ergot alkaloids. These ergot alkaloids are produced by the endophytic fungus, which can be found universally within the plant, but are primarily concentrated in the seed head (Fribourg H.A. 1991b). The most common ergot alkaloid found in tall fescue is ergovaline (Yates *et al.* 1985), but other ergot alkaloids produced include ergonovine, ergotamine, and lysergic acid, which are thought to contribute to fescue toxicosis as well (Klotz *et al.* 2007).

Ergovaline is known to mimic the effects of dopamine, sometimes referred to as prolactin inhibiting hormone, and is considered a dopamine agonist (Larson *et al.* 1995). Ergot alkaloids bind to dopamine receptors, which in turn increase circulating levels of dopamine (Strickland *et al.* 2011). High concentrations of dopamine or ergovaline cause a decrease in prolactin concentrations. These hormonal imbalances affect maturation, growth, and reproduction, the most common symptoms of fescue toxicosis (Paterson *et al.* 1995; Strickland *et al.* 2011).

Tall Fescue Toxicosis

Cattle that graze endophyte infected (E+) tall fescue exhibit signs commonly referred to as fescue toxicosis. This is not only a major concern in

cattle but in sheep and horses as well. With tall fescue covering over 35 million acres in the United States and over 20% of the beef cattle herd grazing tall fescue (West & Waller 2007), it was previously estimated to equate to a loss of over $600 million in annual revenue to the beef industry from fescue toxicosis alone (Hoveland 1993). However, with the addition of horses and other small ruminants, such as sheep, this loss is estimated to be over $1 billion (Strickland *et al.* 2011).

Two issues that are not as widely discussed when it comes to tall fescue toxicosis are fat necrosis and fescue foot, both are caused by the consumption of E+ tall fescue. Fat necrosis is described as necrotic fat deposits in differing shapes and sizes in the mesentery of the abdominal cavity of cattle grazing E+ tall fescue (Smith *et al.* 2004). The most severe but least observed effect of E+ tall fescue is fescue foot. Hyperemia, accompanied by severe swelling, occurs at the coronary band, between the dewclaw and hooves (Hemken *et al.* 1984); this often times leads to gangrene of the animals extremities (Lyons *et al.* 1986).

Tall fescue toxicosis is not a definitive disease; as a result, there is not a specific test to determine whether an animal has it or not. In beef cattle, ergovaline is known to cause reduced feed intake, weight gain, reproductive efficiency, serum prolactin concentrations, and milk production; retained hair coat, and peripheral vasoconstriction are observed as well (Hemken *et al.* 1984; Fribourg H.A. 1991a; Strickland *et al.* 2011). These phenotypes are common symptoms of cattle suffering from fescue toxicosis, which is sometimes referred

to as “summer slump”. These symptoms have been shown to be more pronounced when an animal is experiencing heat stress, in temperatures greater than 32^{o}C (Hemken *et al.* 1981), but are known to also be observed at temperatures below that point (Parish *et al.* 2003). The effects of fescue toxicosis vary between animal and levels of consumption of the toxin within the plant (Fribourg H.A. 1991a).

Fescue Toxicosis and Reproductive Depression

Beef cattle production is one of the top agriculture commodities in the midsouth United States, with most of the beef cattle raised coming from cow - calf operations. Many processes are affected by fescue toxicosis but one of the most notable is a decrease in reproductive efficiency. Cattle, sheep and horses are affected, to varying degrees, with poor conception rates and other reproductive issues due to the consumption of endophyte infected tall fescue when compared to animals that are not grazing endophyte infected tall fescue. Each of the aforementioned species experiences different levels of severity when it comes to fescue toxicosis; this adds an even greater level of complexity to the disease.

Mares in foal are known to have prolonged gestation lengths, dystocia, agalactia, and weakened foals while grazing infected tall fescue (Cross *et al.* 1995). The effect of fescue toxicosis is unique for mares because they are completely remedied by the removal of fescue from the mare’s diet 30 days prior to parturition, and no lingering side effects are observed. There has also

been successful treatment with the dopamine antagonist, domperidone, to eliminate negative effects from consumption of infected tall fescue (Cross *et al.* 2012).

Sheep grazing endophyte-infected tall fescue are not as severely affected as cattle; however, they do still experience reduced reproductive efficiency, circulating prolactin, and cholesterol (Bond *et al.* 1988). Ewes have been shown to have delayed conception rates after exposure to a ram while grazing E+ tall fescue. They also experience lowered fertility but have a normal feed intake and growth rate (Porter & Thompson 1992).

Similar to other symptoms of fescue toxicosis, the exact way in which reproduction in cattle is affected is inconsistent and not clearly understood. Strickland *et al.* (2011) suggested that there is an interaction between environmental effects (e.g., ambient temperature, length of exposure time, and nutritional management) and other factors such as genetics and age.

The most important aspect of a cow-calf operation is yearly calf production. Without a dam producing a calf yearly, producers lose income on that cow. Calf production can be thought to begin when a heifer hits puberty. Washburn *et al.* (1989) reported that heifers raised on E+ tall fescue had delayed onset of puberty and decreased first-service pregnancy rates. However, Bolt and Bond (1989) demonstrated that heifers grazing E+ tall fescue had normal estrous cycle activity. Ahmed *et al.* (1990) also reported that heifers grazing E+ tall fescue were cycling and ovulating normally, but that the corpus

luteum function may have been altered from ergot alkaloid consumption hindering the maintenance of pregnancy. Progesterone is a hormone, secreted by the corpus luteum, needed for the establishment and maintenance of pregnancy. It has been reported that serum progesterone concentrations have been reduced in heifers consuming E+ tall fescue but the use of a dopamine antagonist returned progesterone concentrations to similar levels as those animals grazing E- tall fescue (Jones *et al.* 2003).

Bulls are affected at varying degrees while grazing E+ tall fescue, but even less is known on the effects of male reproduction losses. It was reported that Angus bulls consuming E+ tall fescue seed tended to have reduced sperm motility when compared with bulls on an E- tall fescue seed diet (Jones *et al.* 2004). During the months of high ambient temperature, Decreased motility and slower velocities were observed in sperm of Brahman-influenced bulls consuming E+ tall fescue as compared to sperm from bulls who were consuming E- tall fescue (Looper *et al.* 2009). There are also several studies that report no alkaloid effect on sperm characteristics consuming E+ tall fescue (Schuenemann *et al.* 2005; Strickland *et al.* 2011; Stowe *et al.* 2013). This is a field that requires further investigation.

Fescue Toxicosis and Growth

Calf birth weights are an important factor when it comes to breeding decisions, but the line between what calves are too big and what calves are too small is a fine one. Small birth weights increase calving ease but larger calves

achieve higher weaning weights. Higher weaning weights generate a larger income for cow-calf producers. Bolt and Bond (1989) reported that calves from dams grazing E+ tall fescue had smaller birth weights compared to calves from dams not on E+ tall fescue. Watson *et al.* (2004) investigated cow-calf pairs productivity grazing E+ tall fescue compared to a nonergot alkaloid-producing strain of tall fescue, and found that the dams grazing E+ tall fescue had lower serum prolactin and gave birth to smaller calves. They also reported that those dams on the nonergot alkaloid-producing tall fescue had higher weaning weights, indicating that ergot alkaloids influence calf phenotypes (Watson *et al.* 2004).

The grazing of E+ tall fescue by cows can lead to loss of calf body weight, slower calf gains, and lighter calves at weaning (Schmidt & Osborn 1993; Paterson *et al.* 1995). Calves weaned from dams grazing E+ tall fescue were lighter than calves weaned from dams grazing E- tall fescue (Schmidt *et al.* 1983; Gay *et al.* 1988; Essig *et al.* 1989; McDonald 1989). Cows grazing E+ tall fescue have decreased milk production compared to cows that are not grazing E+ tall fescue (Ashley *et al.* 1987; Keltner *et al.* 1988; Peters *et al.* 1992). Peters *et al.* (1992) reported that cows grazing E+ tall fescue had a reduction in milk production by 25% compared to cows grazing E- tall fescue or orchardgrass pastures. It is thought that this reduction in milk production influences calf weaning weight.

Fescue Toxicosis and Serum Prolactin Depression

Prolactin is a protein hormone that is secreted from the anterior pituitary gland and is predominantly involved with lactation and mammary gland development (Riddle *et al.* 1933). More recent research has estimated that prolactin may be involved in as many as 300 different functions (Ben-Jonathan *et al.* 1996). It is primarily inhibited by dopamine; therefore the dopaminergic action of ergovaline has the potential to suppress prolactin release (Cross *et al.* 1995). In cattle a decrease in serum prolactin concentrations is consistently noted as a symptom of fescue toxicosis (Fribourg H.A. 1991b; Parish *et al.* 2003).

Heat stress is prevalent in cattle raised in the mid- south and impacts many physiological systems, specifically reproduction (Jordan 2003). For animals to effectively dissipate extra heat in the summer months, they must shed their rough, winter coats for smooth, summer coats. Cattle that cannot shed their winter hair coat retain heat, which increases the overall core temperature of the body, affecting different physiological systems (Turner & Schleger 1960). As day length increases, prolactin naturally increases; this increase in prolactin causes an animal to shed their winter coats (Leining *et al.* 1979). Animals consuming E+ tall fescue experience a decrease in prolactin due to the ergot alkaloids ability to mimic dopamine. The retention of a rough hair coat in summer is a distinctive sign of fescue toxicosis (Nihsen *et al.* 2004; McClanahan *et al.* 2008).

Dopamine Antagonists

Dopamine is known to inhibit prolactin secretion. A reduction in serum prolactin has been demonstrated numerous times as consequence of grazing E+ tall fescue when compared to animals not grazing E+ tall fescue (Fribourg H.A. 1991b; Parish *et al.* 2003). Ergot alkaloids are known dopamine agonists, therefore choosing a dopamine antagonist as therapy to alleviate the effects of fescue toxicosis is a logical choice. Henson *et al.* (1987) administered spiperone, a known dopamine antagonist, intravenously to wethers consuming E+ tall fescue and found that it decreased dopamine concentration while increasing prolactin concentrations. Similarly, the use of the dopamine antagonist metoclopramide increased concentrations of circulating prolactin in steers grazing E+ tall fescue, and metoclopramide-treated steers also spent more time grazing (Lipham *et al.* 1989).

More recently domperidone, another dopamine antagonist, increased weight gains, as well as maintained levels of circulating progesterone when administered to heifers consuming E+ diets (Jones *et al.* 2003). Domperidone eliminates the effects of fescue toxicosis when given to pregnant mares (Cross *et al.* 2012).

Alternative Management

Farmers have attempted to overcome the effects of fescue toxicosis in cattle by using alternative practices. Initially, producers attempted to reseed with a tall fescue stand that did not contain the endophyte. Without the

presence of the endophyte, cattle did not experience the symptoms of fescue toxicosis but the plant's tolerance to disease, drought, and heavy grazing was severely compromised (Read & Camp 1986). Cultivars were developed using a novel endophyte-infected tall fescue stand to reseed pastures. The novel endophyte still allowed for the beneficial resistance to disease, drought, and heavy grazing, but improved daily weight gains and prolactin concentrations in lambs (Bouton *et al.* 2002). The process to kill off E+ tall fescue and reseed with another forage can be timely and costly, but economic analysis has indicated that if a pasture has an infection rate >70%, pasture replacement may be a more profitable route when compared to retaining E+ tall fescue stands (Zhuang *et al.* 2005).

Another cost-effective option producers utilize is interseeding with a dilution crop. A dilution crop does not require the complete removal of tall fescue from pastures. Clover interseeded within the pasture can help diminish the effects of fescue toxicosis and has been shown to improve the performance of steers (McMurphy *et al.* 1990) and cow-calf pairs (Hill *et al.* 1979). This is thought to only increase gains because of an improved quality of diet (Aiken & Strickland 2013). White clover (*Trifolium repens L.*) is the predominant legume seeded with tall fescue but red clover (*Trifolium pretense L.*) is also used (Lusby *et al.* 1990).

Replanting is not always a feasible option. Improved management practices are a cost effective way for producers to mitigate the effects of fescue

toxicosis without having to reseed entire pastures. Tall fescue is a productive forage option, but can be detrimental to animal gains in the summer months. For producers with available land, a viable option would be to remove cattle from tall fescue pastures during the summer months. Steer gains have been shown to improve due to the rotation of animals from a tall fescue pasture to a summer pasture that does not include E+ tall fescue (Aldrich *et al.* 1990).

Moving cattle off of tall fescue is not always an option. Producers can also utilize a higher stocking density to overgraze fields (Bransby *et al.* 1988). This increases grazing pressure which keeps the forage at a more immature production stage. Another suggested method is to cut tall fescue before the seed head develops (Roberts & Andrae 2004). There are dual benefits to these methods. Ergovaline is primarily located in the seed head so this cuts down on the toxicity levels ingested (Fribourg H.A. 1991b). Also, immature tall fescue is leafier, which provides better quality forage to graze. This is not only a cost effective process for many producers, but keeps the tall fescue in a shorter growth stage, providing a more beneficial and productive forage.

Genetic Resistance

Fescue toxicosis, as mentioned previously, is not a definitive disease. As with most desirable and important traits in agriculture there is not one single gene that is responsible for the effects of fescue toxicosis, indicating the response to E+ tall fescue exposure can be considered a complex or quantitative trait. This complex nature makes overcoming the symptoms of

fescue toxicosis a challenge. With the cost and problems associated with altering the environment, there has been more of a focus on taking a genetic approach to overcoming the symptoms of fescue toxicosis.

Mice have been used as models for tall fescue toxicosis studies, with certain lines of mice being selected as either resistant or susceptible. In a study by Hohenboken and Blodgett (1997), mice were selected for eight generations for either resistance or susceptibility based on their growth when fed E+ tall fescue seed. Growth in the resistant line was not retarded and the enzymes involved in detoxification reactions were higher than in the susceptible line (Hohenboken & Blodgett 1997).

The Dopamine Receptor D2 (DRD2) gene is located on the bovine chromosome 15 and has been shown to play a role in prolactin secretions (Civelli *et al.* 1993). The 'A' allele found at nucleotide position 534 in the DRD2 gene was shown to be associated with increased serum prolactin concentrations and decreased hair coat score in Angus cattle grazing E+ tall fescue (Campbell *et al.* 2013).

A genome wide association study (GWAS) was performed using an Illumina 50k bovine single nucleotide polymorphism (SNP) chip. This was to identify other possible SNP that may be associated with differential sensitivity to fescue toxicosis in beef cattle. The results of this study identified 24 significant SNP in the bovine genome associated with either adjusted birth weight (ABW) or adjusted 205-day weight (A205). The subjects used for this

study were split into two groups based on performance. One group was high-performing (n=24) and the other low-performing (n=24) cows with calves. These determinations were based on the phenotypes; number of calves produced and A205. The phenotypes, ABW and A205 were chosen as response variables to be statistically analyzed for significant SNP association. These phenotypes were selected for two reasons: they have been adjusted for variation due to the age of dam and the sex of calf, and they give an unbiased comparison (BIF 2010). Two of these SNP, found to be associated with A205, were in an exon portion of the gene XK, Kell blood group complex subunit-related family, member 4 (XKR4) (Campbell 2012).

XKR4 is located on BTA 14 in cattle, and is sparsely represented in the literature. This is primarily due to the fact that not much is known about this gene. In humans, the absence of this red blood cell membrane protein is associated with McLeod syndrome, a genetic disorder that affects multiple systems (Danek *et al.* 2001). A study by Jung *et al.* (2007) has indicated that schizophrenia is a manifestation of McLeod syndrome. Iloperidone is an atypical antipsychotic known to have a high DRD2 binding affinity. It is similar to domperidone in that it is a dopamine antagonist (Kalkman *et al.* 2001). A study by Lavedan *et al.* (2009) suggests that a SNP upstream of the XKR4 gene is associated with efficacy in treating schizophrenia patients using iloperidone. Iloperidone acts in the same manner as domperidone, indicating that XKR4 could potentially mediate the effects of fescue toxicosis.

Few studies involving XKR4 have been conducted in cattle. A study by Porto Neto *et al.* (2012) implies an association between rump fat thickness and 3 SNP (rs42646645, rs42646708, and rs41724387) in the XKR4 gene. These SNP were found to be in linkage disequilibrium, *D'* = 1.00. Another study suggests strong associations with the XKR4 genotype and residual feed intake, average daily feed intake, and average daily gain (Lindholm-Perry *et al.* 2012). Fescue toxicosis reduces cattle weight gain, and a genetic approach to mitigate these effects could be beneficial. The most recent study indicated an association between a SNP (rs42646708) in the XKR4 gene and serum prolactin concentrations in Tennessee beef cattle (Bastin *et al.* 2014). These findings suggest animals with the 'A' allele have increased serum prolactin concentrations (Bastin *et al.* 2014). Reductions in these phenotypes are commonly seen in cattle experiencing the effects of fescue toxicosis.

Summary

Tall fescue is the most common forage for beef production utilized in the mid-south United States. It is an ideal forage for many producers due to its range of positive attributes. However, there are drawbacks in using this forage. When animals consume E+ tall fescue, they develop symptoms commonly referred to as fescue toxicosis. Removal of tall fescue forage is a costly and time consuming endeavor, but the development of genetic selection techniques may be a beneficial route to mitigate the effects of fescue toxicosis.

Our objective was to evaluate genotype associations with phenotypes of economic importance in cow-calf herds grazing endophyte-infected tall fescue in Missouri. These phenotypes included serum prolactin concentration, hair coat score, body condition score, adjusted birth weight, and adjusted 205-day weight. In the first experiment, we examined the effects of dam genotype on serum prolactin concentration, hair coat score, and body condition score. In the second experiment, we examined the effects of dam and calf genotype on adjusted birth weight and adjusted 205-day weight.

Over five years of well-kept calf data were recorded and analyzed for the key factors adjusted birth weight and adjusted-205 day weight. Calves and dams were then genotyped for SNP in the DRD2 and XKR4 genes and associations were analyzed. If associations between genotype and phenotype are found, a genetic marker panel could be developed to select replacement heifers and sire bulls, leading to a calving herd that is more resistant to the negative effects of grazing E+ tall fescue.

Chapter 2: An evaluation of DRD2 and XKR4 on Missouri Fall Calving Beef Cattle Herds

Abstract

Decreased growth, reproduction efficiency, lactation, and serum prolactin concentrations can be observed in cattle suffering from the effects of fescue toxicosis. These symptoms are estimated to cause the beef industry over $600 million in losses annually. Single nucleotide polymorphisms (SNP) in the Dopamine Receptor D2 (DRD2) and XK, Kell blood group complex subunit related family member 4 (XKR4) genes of the dam and calf were assessed for their ability to be informative for serum prolactin concentration, hair coat score (HCS), body condition score (BCS), adjusted birth weight (ABW) and adjusted 205-day weight (A205) in fall calving beef cattle grazing endophyte infected tall fescue in Missouri. The results indicated that dam genotypes at DRD2 and XKR4 combined were associated with serum prolactin concentrations ($p = 0.04$). Serum prolactin concentration was increased when cows had 2 copies of the 'A' allele at DRD2 and at least 1 'G' allele at XKR4. Dam genotype at DRD2 was associated with HCS and BCS ($p = 0.0024$ and $p = 0.011$, respectively) such dams with 'AG' or 'AA' genotype had decreased HCS and dams with the 'AG' genotype had decreased BCS. Dam genotype at DRD2 and XKR4 was associated with ABW ($p < 0.0001$ and $p = 0.027$, respectively), such that dams with the 'AG' genotype at DRD2 and 'GG' genotype at XKR4 had increased ABW. Calves with the 'AA' genotype at XKR4 were associated with increased A205 ($p = 0.011$). These results suggest that genetic selection using these markers could improve cow-calf production in beef cattle grazing endophyte-infected tall fescue.

Introduction

Tall fescue (*Lolium arundinaceum Schreb.*), a cool-season perennial bunch grass, is the most widely used forage in the southeastern United States (Stuedemann J.A. 1988). Tall fescue is popular with livestock producers due to its quick establishment and ability to withstand drought and over-grazing. The resilience of tall fescue is partly due to a symbiotic relationship with an endophytic fungus (*Neotyphodium coenophialum*). The endophyte produces ergot alkaloids - primarily the dopamine agonist ergovaline - which when ingested by cattle has many negative side effects. Some of these side effects include decreased reproductive efficiency, vasoconstriction, reduction in dry matter intake, and in extreme cases, necrosis of the extremities (Fribourg H.A. 1991a). Lactation has also been shown to be compromised, thought to be either due to reduced feed intake or decreased serum prolactin (Porter & Thompson 1992). Collectively these symptoms are often referred to as fescue toxicosis and costs the beef industry more than $600 million annually (Strickland *et al.* 2011).

Previous research on beef cattle in Tennessee has shown that a single nucleotide polymorphism (SNP) (rs41749780) in the Dopamine receptor D2 (DRD2) gene influences serum prolactin concentrations in beef cattle grazing E+ tall fescue (Campbell *et al.* 2013). Decreased serum prolactin concentrations are commonly noted as a primary indicator of fescue toxicosis (Fribourg H.A. 1991b; Strickland *et al.* 2011). Domperidone, a dopamine antagonist, has been

shown in horses to reduce the effects of fescue toxicosis in pregnant mares grazing endophyte-infected tall fescue (Cross *et al.* 2012). Dopamine D2 antagonism increases prolactin secretion (Ben-Jonathan & Hnasko 2001). Campbell *et al.* (2013) found an association with the 'A' allele of rs41749780 and increased prolactin concentrations in cattle grazing endophyte infected tall fescue.

Campbell (2012) utilized a 50k SNP chip to perform a Genome Wide Association Study (GWAS) to discover other possible SNP that could be associated with resistance to fescue toxicosis. The cows that were used for this study were chosen based off of their performance records as either high-producing or low-producing dams. The response variables chosen were calf adjusted birth weight (ABW) and calf adjusted 205-day weight (A205). The results of the GWAS indicated 24 SNP associated with either ABW or A205. Of those 24, two SNP were found to be associated with A205 in the XK, Kell blood group complex, subunit-related family, member 4 (XKR4) gene.

An association has been noted between the XKR4 genotype and efficacy of schizophrenia treatment using iloperidone, a dopamine antagonist related to domperidone (Lavedan *et al.* 2009; Fijal *et al.* 2012). Treatment with iloperidone is associated with an elevation in prolactin (Jain (2000); Cutler et al. (2008)). Three SNP in the XKR4 gene (rs42646645, rs42646708 and rs41724387) were found to be associated with subcutaneous rump fat in beef cattle (Porto Neto *et al.* 2012). Bastin *et al.* (2014) found an association with the 'A' allele of

rs42646708 and increased prolactin concentrations in cattle grazing endophyte infected tall fescue.

The purpose of this study was to examine the possible effects of DRD2 and XKR4 genotype, on serum prolactin concentration, hair coat score, body condition score, A205 and ABW on a large herd of beef cattle grazing endophyte-infected tall fescue in Missouri.

Materials and Methods

Data Set

The cattle herd at Southwest Research Center (SWC) located in Mt. Vernon, Missouri is a primarily Angus based fall-calving herd. There are approximately 143 dams with 5 years of phenotypic calving data. This herd primarily grazes Kentucky-31 tall fescue throughout the natural growing period but is fed stockpiled tall fescue hay throughout the winter months. Cows are removed from this herd if there is failure to re-breed after one round of artificial insemination followed by 30 days of exposure to a clean-up bull.

Serum collection

Blood samples were collected via jugular venipuncture from dams (n=100) in June 2013. Blood samples were centrifuged for 15 min at 10,000 RPM and serum samples were stored frozen (-20^{o}C) for determination of serum prolactin concentrations as described by Bernard *et al.* (1993). The intra-assay CV was 7.35% and inter-assay CV was 4.05%. Hair coat scores were assessed

(BIF 2010), with a score of 1 indicating a smooth, slick coat and a score of 5 indicating a rough, retained dead hair coat. Body condition scores were also assessed, with a score of 1 indicating an emaciated, unlivable condition and a score of 9 indicating an obese, fat-laden individual (BIF 2010).

Genotyping

Hair samples from the tail switch of dams (n=143) and calves (n=501) were collected for DNA extraction from 2008 to 2013. DNA was extracted using Quickextract from 5-10 follicles (Epicentre, Madison, WI). Custom Taqman Assays (Applied Biosystems, Foster City, CA) were previously designed and verified for each SNP to genotype each cow and calf (Table 1). Fluorescent reporter dyes were quantified using a 7900HT Fast Real Time PCR System (Applied Biosystems, Carlsbad, CA).

Statistical Analysis

The SNP rs41749780 in DRD2, rs42646645 and rs42646708 in XKR4 all yield the genotypes 'AA', 'AG', and 'GG'. Linkage disequilibrium analysis was run between rs42646645 and rs42646708, using the genetics package in R, resulted in D' = 0.99. The 'G' allele from rs42646645 is linked with the 'A' allele of rs42646708. A greater percentage of animals were genotyped for rs42646645 in XKR4; therefore, only these results are presented.

All statistical models were fit using the number of beneficial alleles present as treatment levels. Mixed model analysis of variance and regression

Table 1. Location and assay information for SNP included in study

SNP	BTA	Position (bp)	Forward Primer Sequence	Reverse Primer Sequence	VIC Reporter Sequence	FAM Reporter Sequence
DRD2[1]	15	404365	TCTGACCTTACCAGGAACAATCCA	CTGAAGCCAGGCTGAGTCT	ACCGGAATCACCC	ACCGGAGTCACCC
XKR4[2]	14	2590669	TGCTCCTCCACAGACAATTCATATG	AGACGGGTGTTAGCACAGAAC	TCTCGCATTCTCC	TCTCGCGTTCTCC
XKR4[3]	14	2667113	CGTCTTGAACAGGATTTCGGTTTTC	AGGCTACCAGATTTTTAGCACTAATGAAG	TGGTTGTAGCTAAAAGC	TGGTTGTAGCTGAAAGC

[1]rs41749780
[2]rs42646645
[3]rs42646708

analyses were performed in SAS 9.3 (Cary, NC). Associations were declared significant at p = 0.05.

A completely randomized design was utilized to evaluate genotype effect on dam prolactin, hair coat score (HCS), and body condition score (BCS). Genotype effects included number of beneficial alleles present for the dam SNP in DRD2 and XKR4 as separate analyses. A log transformation was applied to prolactin values, and back transformed least squares means are reported. To evaluate the interactions between genotypes at DRD2 and XKR4, a regression model was chosen applying a response surface design. This allowed for a visualization of the combined effect the genotypes had on the response variables.

For calf phenotype, response variables adjusted birth weight (ABW) and adjusted 205-day weight (A205), were evaluated as a randomized block design with fixed effects of dam genotype, calf genotype and their interaction, and year as a random effect. These were determined using the formulas below (Equation 1 and 2 respectively) as described by the Beef Improvement Federation (BIF 2010) with the adjustments for age of dam listed in Table 2.

Equation 1

$$Adj\ Birth\ Wt = \ Birth\ Weight\ (BW) + Dam\ Age\ Adj.$$

Equation 2

$$Adj\ 205\ WW = \frac{Actual\ WW - Birth\ Weight\ (BW)}{Age\ at\ Weaning\ in\ Days} \times 205 + BW + Dam\ Age\ Adj.$$

Table 2. Standard age of dam adjustment factors (kg) for adjusted birth weight and adjusted 205-day weaning weight[1]

Age of Dam (Years)[2]	Birth Weight Adjustment Factor	Weaning Weight Adjustment Factor	
		Male	Female
2	3.64	27.27	24.54
3	2.27	18.18	16.36
4	0.90	9.09	8.18
5-10	0.00	0.00	0.00
11 & Older	1.36	9.09	8.18

[1]Beef Improvement Federation (2010)
[2]Age of dam at calving

Results

Prolactin

SNP genotype of the DRD2 and XKR4 genes were not associated with serum prolactin concentrations in dams grazing E+ tall fescue (p=0.61 and p=0.12, respectively). A response surface model (Figure 1) showed there was an additive effect of both genotypes at DRD2 and XKR4 on serum prolactin concentrations. Serum prolactin concentrations were greater when there were 2 'A' alleles for DRD2 and at least 1 'G' alleles for XKR4 (p=0.04). The overall model fit was $r^2 = 0.10$.

Hair Coat Score

Genotype at DRD2 was associated with HCS (p=0.0024). Animals that had 0 'A' alleles had higher HCS than those with 1 or 2 'A' alleles. Genotype at the XKR4 gene was not associated with HCS (p=0.28), and there was no observed interaction between DRD2 and XKR4 genotype and HCS (p=0.79) (Table 3).

Body Condition Score

Animals with only 1 copy of the 'A' DRD2 allele had lower BCS than animals possessing either 0 or 2 copies of the 'A' allele (p=0.011). Genotype at XKR4 was not associated with BCS (p=0.63), and there was no interaction noted between DRD2 and XKR4 genotypes (p=0.44).

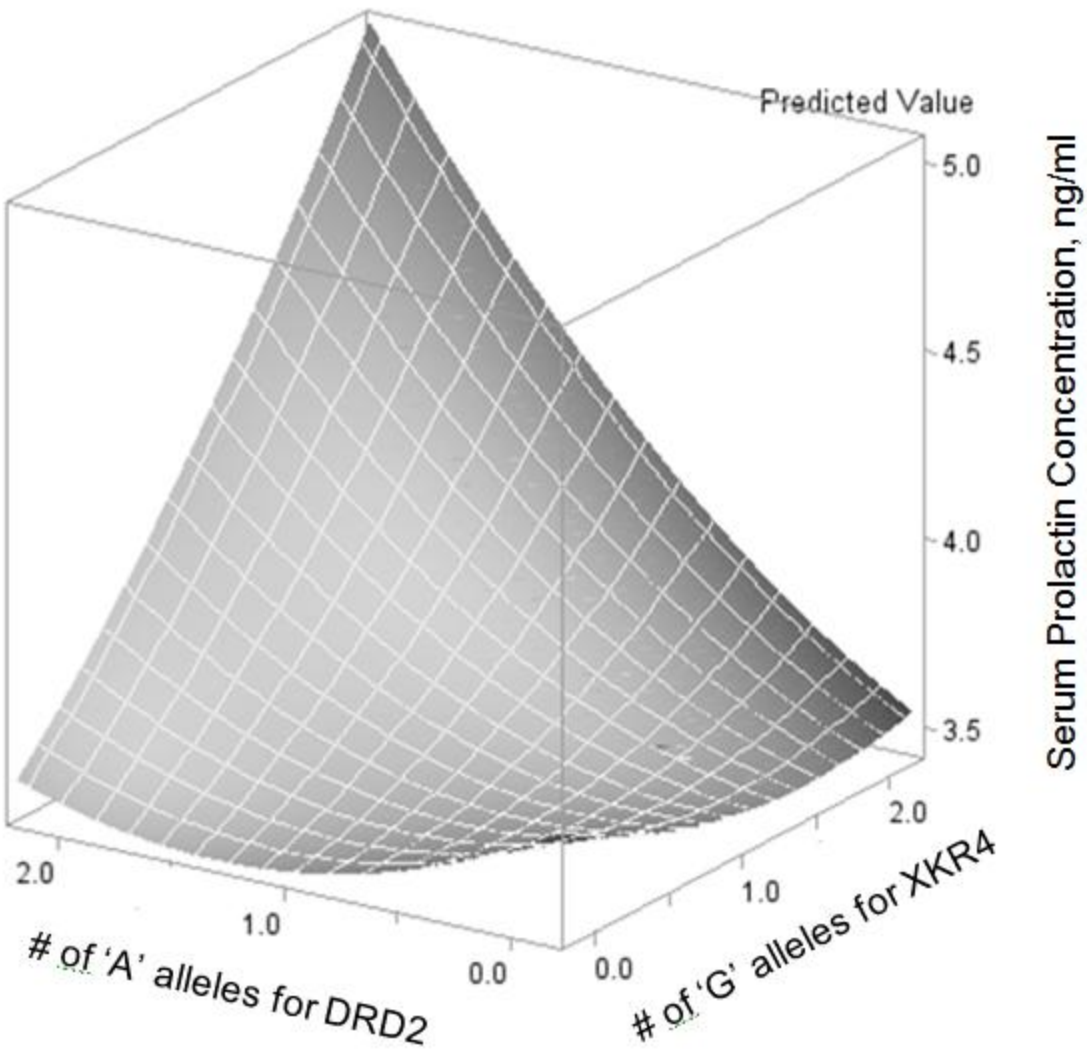

Figure 1. Response surface for Prolactin

Table 3. Dam genotype effect on Serum Prolactin Concentration (PRL), Hair Coat Score (HCS), and Body Condition Score (BCS)

	Dam DRD2			Dam XKR4			Additive effect?[5]
	GG Mean	**AG Mean**	**AA Mean**	**GG Mean**	**AG Mean**	**AA Mean**	
PRL[2]	$35.4^{A,1}$	35.3^{A}	40.5^{A}	79.5^{A}	40.1^{A}	35.0^{A}	Yes
p-value		0.61			0.12		0.04
HCS[3]	2.98^{A}	2.19^{B}	2.24^{B}	1.38^{A}	2.41^{A}	2.52^{A}	No
p-value		0.0024			0.28		0.79
BCS[4]	6.25^{A}	5.86^{B}	6.23^{A}	6.50^{A}	6.12^{A}	6.09^{A}	No
p-value		0.011			0.63		0.44

[1] Superscript letters denote mean differences with gene ($p < 0.05$) by Fisher's LSD

[2] back-transformed least squares means associated with genotype, ng/mL

[3] Scored 1 to 5 (1= slick summer coat, 5 = full winter coat) BIF (2010)

[4] Scored 1 to 9 (1= extremely thin, 9 = extremely obese) BIF (2010)

[5] Additive effect of response model for dam genotype for DRD2 and XKR4

Effect of Dam and Calf Genotype on Calf Phenotype

The dam's DRD2 genotype was associated with her calf's ABW. Dams with 1 'A' allele produced heavier calves than those possessing 0 or 2 copies of the allele (p = 0.029) (Table 4). The dams with 2 copies of the 'G' allele at XKR4 had heavier calves than dams with only 1 or 0 copies of the allele (p=0.027). There was no interaction effect between dam genotype at DRD2 and XKR4 for ABW (p=0.56). Calf genotype at DRD2 or XKR4 was not associated with ABW (p=0.52 and p=0.34, respectively). Combined calf genotype at DRD2 and XKR4 was not associated with ABW (p=0.10). There was no interaction effect between dam genotype and calf genotype at DRD2 for ABW (p=0.91) (Table 5). There was also no interaction between dam and calf XKR4 genotype for ABW (p=0.30) (Table 6)

There was no association for dam genotype at DRD2 or XKR4 on A205 (p=0.22 and p=0.21, respectively) (Table 4). There was no interaction observed for dam genotype at DRD2 and XKR4 for A205 (p=0.84). Calf genotype at DRD2 was not associated with A205 (p=0.90), but calves with 0 copies of the 'G' allele at XKR4 had significantly higher A205 when compared to calves with 1 or 2 copies (p=0.011). There was no effect of calf DRD2 and XKR4 combined on A205 (p=0.78). There was a tendency for an interaction between dam and calf DRD2 genotype (p=0.06) (Table 7). A trend was observed such that A205 tended to be highest when the dam carried 2 DRD2 'A' alleles and the calf carried 1 DRD2 'A' allele, while A205 tended to be lowest when both the dam

Table 4. Dam and calf genotype effect on calf phenotypes Adjusted Birth Weight (ABW) and Adjusted 205-day Weight (A205)

	DRD2 Genotype Means			XKR4 Genotype Means			Additive effect?[4]
Dam genotype	GG	AG	AA	GG	AG	AA	
Adjusted birth weight (kg)[1]	37.2A[B,3]	39.1[A]	35.9[B]	44.7[A]	37.8[B]	37.2[B]	No
p-value		0.029			0.027		0.56
Adjusted 205-day weight (kg)[2]	224.6[A]	217.6[A]	227.9[A]	215.8[A]	214.8[A]	224.8[A]	No
p-value		0.22			0.21		0.84
Calf Genotype	GG	AG	AA	GG	AG	AA	
Adjusted birth weight (kg)	38.4[A]	37.1[A]	38.0[A]	41.7[A]	37.2[A]	37.8[A]	No
p-value		0.52			0.34		0.10
Adjusted 205-day weight (kg)	222.8[A]	223.0[A]	220.1[A]	197.3[B]	214.5[B]	227.7[A]	No
p-value		0.90			0.011		0.78

[1] See Equation 1. Beef Improvement Foundation (2010)

[2] See Equation 2. Beef Improvement Foundation (2010)

[3] Superscript letters denote mean differences within a gene ($p < 0.05$) by Fisher's LSD

[4] Additive effect of response surface model for dam genotype or calf genotype for DRD2 and XKR4 combined

Table 5. Dam-calf genotype interactions for ABW for DRD2

		Dam DRD2 Genotype			p-value[3]
		GG	**AG**	**AA**	
Calf DRD2 Genotype	**GG**	$37.8^{A,1,2}$	39.0^{A}	–	0.91
	AG	36.8^{A}	39.0^{A}	35.6^{A}	
	AA	–	39.1^{A}	36.4^{A}	

[1] Superscript letters denote mean differences ($p < 0.05$) by Fisher's LSD

[2] least squares means associated with genotype, kg

[3] Dam-calf genotype interaction for DRD2 on adjusted birth weight

Table 6. Dam-calf genotype interactions for A205 for DRD2

		Dam DRD2 Genotype			p-value[3]
		GG	AG	AA	
Calf DRD2 Genotype	GG	226.4[A,1,2]	219.2[A]	–	0.06
	AG	223.1[A]	212.1[A]	236.4[A]	
	AA	–	223.1[A]	214.2[A]	

[1] Superscript letters denote mean differences ($p < 0.05$) by Fisher's LSD

[2] least squares means associated with genotype, kg

[3] Dam-calf genotype interaction for DRD2 on adjusted 205-day weight

Table 7. Dam-calf genotype interactions for ABW for XKR4

		Dam XKR4 Genotype			p-value[3]
		GG	AG	AA	
Calf XKR4 Genotype	GG	37.5[A,1,2]	39.0[A]	–	0.30
	AG	36.6[A]	36.8[A]	42.6[A]	
	AA	–	38.3[A]	55.4[A]	

[1] Superscript letters denote mean differences ($p < 0.05$) by Fisher's LSD

[2] least squares means associated with genotype, kg

[3] Dam-calf genotype interaction for XKR4 on adjusted birth weight

and calf carried 1 'A' allele or both carried 2 'A' alleles. There was no interaction between calf and dam genotype for XKR4 (p=0.99) (Table 8).

Discussion

Dam genotype for DRD2 and XKR4 was not associated with serum prolactin concentrations. Campbell *et al.* (2013) reported that the 'A' allele of rs41749780 in DRD2 was associated with increased serum prolactin concentration in the months of April and May. Bastin *et al.* (2014) reported that rs42646708 in XKR4 was associated with increased serum prolactin concentrations in beef cattle grazing E+ tall fescue. The combination of high ergot alkaloid presence as well as an increase in ambient temperature experienced in the month of June, which ranged between 27°C to 37°C with varying amounts of humidity, could have overwhelmed any effect associated with individual genotype. However, having 2 'A' alleles of rs41749780 in DRD2 and at least 1 'G' allele in rs42646645 in XKR4 was associated with increased serum prolactin concentrations. This could indicate that when ambient temperatures are elevated, the genotype at one locus is not influential enough to alter prolactin levels, but the combined genotype at the DRD2 and XKR4 loci may have an effect.

Hair coat score (HCS) was associated with the 'A' allele in DRD2, such that animals with 1 or 2 copies of the 'A' allele had decreased HCS compared to animals with 0 copies of the 'A' allele. The genotype for XKR4 was not associated with HCS and we did not observe any interaction between SNP in

Table 8. Dam-calf genotype interactions for A205 for XKR4

		Dam XKR4 Genotype			p-value[3]
		GG	AG	AA	
Calf XKR4 Genotype	GG	228.3[A,1,2]	223.1[A]	–	0.99
	AG	215.5[A]	211.6[A]	219.3[A]	
	AA	–	195.7[A]	202.7[A]	

[1] Superscript letters denote mean differences ($p < 0.05$) by Fisher's LSD

[2] least squares means associated with genotype, kg

[3] Dam-calf genotype interaction for XKR4 on adjusted 205-day weight

these two genes. The results for DRD2 are similar to what has been previously seen by Campbell *et al.* (2013), who reported that animals grazing E+ tall fescue in the month of June with the 'AA' and 'AG' genotype had lower HCS than animals with the 'GG' genotype.

The 'AG' genotype at DRD2 was associated with lower body condition score (BCS) than observed in animals with 'AA' or 'GG' genotypes. No association was observed between BCS and XKR4 genotype or the interaction between DRD2 genotype and XKR4 genotype. No previous report has investigated the relationship between BCS and genotype at DRD2 or XKR4. Body condition score is of course a highly polygenic quantitative trait subject to both permanent and temporary environmental influences, including consumption of E+ tall fescue.

Dam 'AG' genotype for DRD2 was associated with heavier calves at birth. Dam 'GG' genotype for XKR4 was also associated with heavier calves at birth. However, dam genotype for DRD2 and XKR4 combined was not associated with ABW. Calf genotype for DRD2 and XKR4 was not associated with its own ABW. Interactions were also compared between dam DRD2 and calf DRD2, as well as dam XKR4 and calf XKR4. None of these combinations were associated with ABW. There has not been any previous research regarding these SNP and calf ABW. However, studies have reported that cows grazing E+ tall fescue give birth to smaller calves (Bolt & Bond 1989; Watson *et al.* 2004). Fall calving dams are in their third trimester, a time for immense fetal growth, when tall

fescue experiences a surge in growth and endophyte presence (Eley *et al.* 1978; Ball *et al.* 1996). Identifying genotypes associated with the effects of fescue toxicosis, such as decreased birth weights, could be used to select for resistance to this disorder.

Dam genotype at DRD2 and XKR4, as well as the combination of the two loci, did not have a significant effect on A205. Calf genotype for DRD2 was also not associated with A205; however, calf 'AA' genotype at XKR4 was associated with increased A205. We did not observe a combined effect for calf genotype at DRD2 and XKR4 on ABW. We observed a trend for an interaction between dam genotype and calf genotype at DRD2. Calves with the 'AG' genotype tend toward increased A205 when dams have two copies of the 'A' allele, but have decreased A205 when the dam only has 1 copy of the 'A' allele or when the dam has the genotype 'AA' and the calf has the genotype 'AA'. As seen with ABW, A205 is decreased in calves whose dams are grazing E+ tall fescue (Keltner *et al.* 1988; Essig *et al.* 1989; McDonald 1989; Paterson *et al.* 1995). It has been speculated that decreased milk production could lead to decreased A205 (Peters *et al.* 1992; Paterson *et al.* 1995). Fall born calves are weaned around May, and so are grazing tall fescue the last couple of months before weaning. The increased A205 could be because of an increased tolerance to the endophyte present during that time, or since they are still consuming milk, calves could have increased A205 due to an increased milk production from the dam. We did not measure milk production of dams in this study.

Most agricultural traits of interest are complex traits and have many variables that control their outcome. This is the reason a GWAS was utilized to identify multiple SNP that could be more informative at alleviating the effects of fescue toxicosis. However, there are several limitations from using the GWAS which might impact the future of this research. The GWAS was performed using a medium density SNP chip and now there are genotyping options that provide a larger number of SNP to be evaluated. We also did not have a large population size to evaluate the genotype-phenotype associations. In the future, we hope to be able to evaluate these SNP on a larger number of animals to increase the overall power of these studies. We only focused on SNP that were found within genes that had known functions. It is possible that other SNP that have significant associations could be more informative for the performance phenotypes of interest. Future work will include a more thorough investigation into the effects and associations of these other SNP on performance traits of interest. Complex traits make finding a simple genetic solution impossible; the amount of variation that can be explained by a single SNP is minimal. However, even with the limitations posed by this GWAS, it is an important tool. Multiple SNP will be required to explain the greatest amount of variation in these phenotypic differences.

Previously, these SNP have only been evaluated in steers and heifers after weaning, and therefore could be affecting different systems within the calf. It would be interesting in the future to evaluate certain other factors to see if they were influencing the allele effect, such as grazing time, feed to gain ratio,

milk yield, or overall genetic effect. These parameters could give a better explanation to the physiological response in the cow calf model.

In the future our goal is to develop a multi-locus marker panel that is commercially available to producers. A genetic test such as this would allow producers to cultivate a breeding herd that is resistant to the effects of fescue toxicosis by having their cows and replacement heifers genotyped or selecting herd sires based off of their ability to pass on desired traits. This could provide producers a more permanent management solution which would lead to greater profitability.

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Vita

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